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LABORATORY AND FIELD EVALUATION OF SS220 AND DEET AGAINST MOSQUITOES IN QUEENSLAND, AUSTRALIA[4]

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ABSTRACT. Laboratory and field efficacy trials comparing deet (*N*,*N*-diethyl-*3*-methylbenzamide) and SS220 [(1S, 2′S)-2-methylpiperindinyl-3-cyclohexen-1-carboxamide] against mosquitoes in Queensland, Australia, were conducted. In the laboratory, both compounds provided between 150 and 195 min of protection against *Aedes aegypti* and between 18 and 80 min of protection against *Anopheles farauti*. In laboratory tests against *Culex annulirostris*, 20% SS220 provided 3 h of protection and 20% deet provided >6 h of protection. A field efficacy test was conducted at Redcliffe, Queensland in January 2008 and the predominant mosquito species collected was *Cx. annulirostris* (84.4% of collection). In the field, 20% SS220 provided significantly better protection against mosquitoes than 20% deet. Seven hours after application, SS220 provided greater than 96.0% protection against all mosquitoes, whereas 20% deet provided 58.9% protection.

KEY WORDS *N*,*N*-diethyl-*3*-methylbenzamide, [(1S, 2′S)-2-methylpiperindinyl-3-cyclohexen-1-carboxamide], mosquito repellents, *Culex annulirostris*, Australia

INTRODUCTION

The use of personal protection measures, particularly the application of topical insect repellents to exposed human skin, has long been advocated to minimize human contact with vector and nuisance mosquitoes (Gupta and Rutledge 1994, Debboun et al. 2007). Deet (*N*,*N*-diethyl-*3*-methyl benzamide) was first marketed commercially in 1956 (McCabe et al. 1954) and has since been widely used in insect repellent products for use on human skin to protect against biting and nuisance mosquitoes (Curtis et al. 1990, Brown and Hebert 1997, Elston 1998, Fradin 1998, Qiu et al. 1998, US Environmental Protection Agency [USEPA] 1998, Fradin and Day 2002). In a number of studies a promising new chiral insect repellent compound, (1S, 2′S)-2-methylpiperindinyl-3-cyclohexen-1-carboxamide, known as SS220, has been shown to be as good as or better than deet (Klun et al. 2003; Carroll et al. 2005; Klun et al. 2006a, 2006b; Carroll et al. 2008). The first laboratory tests with this new chemical showed that it was effective against *Aedes aegypti* (L.) and *Anopheles stephensi* Liston (Klun et al. 2003). Additional laboratory trials were conducted against 2 strains of *Anopheles albimanus* Wiedermann and *Ae. aegypti*, and these showed that SS220 provided less protection against *An. albimanus* than the same concentrations of deet (Klun et al. 2004). Recent trials have shown SS220 to be effective against *Phlebotomus papatasi* Scopoli, a vector of Leishmaniasis (Klun et al. 2006a), and the lone star tick, *Amblyomma americanum*, (L.) in simulated field tests (Carroll et al. 2008).

In this article we report laboratory and field test studies to compare the repellent efficacy of SS220 and deet against mosquitoes in southeastern Queensland, Australia.

MATERIALS AND METHODS

Test mosquitoes

Female mosquitoes used in the laboratory evaluation study were *Ae. aegypti* (Townsville strain, originally colonized in 2003), *Anopheles farauti* Laveran (Rabaul strain, colonized in 1965) and *Culex annulirostris* Skuse (Townsville strain, colonized in 1981), and were reared and maintained in the Australian Army Malaria Institute (Frances et al. 2005). Adult mosquitoes used for experimentation were nulliparous females aged between 6 and 9 days old. They were maintained in a photoperiod of 12:12 (L:D) h and tested at 26°C ambient air temperature and 60–70% relative humidity and were provided only water for at least 24 h before testing.

Test repellents

The following repellent compounds were tested: deet (~97% pure), Fluka Chemika, Steinheim, Germany), and SS220, a US De-

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[4] Mention of a commercial product does not constitute an endorsement of the product by the Australian Defence Force, the United States Department of Agriculture, or the United States Department of Defense.

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partment of Agriculture piperidine compound, which was synthesized to >99% chemical purity at the Invasive Insect Biocontrol and Behavior Laboratory (Beltsville, MD; Klun et al. 2003). A 20% solution (V/V) of each chemical in absolute ethanol was used in laboratory and field tests.

Field study site

Field trials were conducted at the edge of a forest at Redcliffe Airport, Redcliffe, Queensland, Australia (153°10′E, 27°23′S), in January 2008. This site is located approximately 30 km north of Brisbane, Queensland. The site is at the edge of native eucalypt and mangrove wetlands, where mosquitoes are commonly found in the summer from September to April each year. The current field trial was conducted when mosquito densities were expected to be high.

Test procedures

Laboratory tests: The methods described by Frances et al. (2005) were used, where a single person (SPF) performed the tests. For each test conducted daily, 100 nulliparous females (6–9 days old) were placed into a screened wire test cage. Tests were conducted by exposing untreated and treated forearms into the test cage containing 100 mosquitoes. A surgical glove was worn during each test to prevent biting on the untreated hand. Each test consisted of 2 parts. In the first part, an untreated forearm was exposed in the cage for 10–30 sec, and the number of probing mosquitoes was recorded as a measure of mosquito feeding avidity. Probing mosquitoes were blown from the arm by the investigator before any blood was taken. Immediately thereafter, the forearm was removed from the cage and 1 ml (ca. 0.43 mg/cm^2 skin) of repellent solution was applied evenly to the same forearm (between the wrist and elbow) with the use of a glove-covered hand. After 2 min of drying, the treated forearm was exposed to the bites of mosquitoes in the same test cage initially for 5 min, then for 5 min at 30-min intervals for *An. farauti* and 60-min intervals for the other species until 3 bites were recorded, whereupon the test was terminated. The tests were terminated after 6 h if 3 bites were not recorded. Between 2 and 5 replicate tests were conducted for each repellent and mosquito species.

Field efficacy test

Three adult volunteers (2 males and 1 female, mean age, 34.3 ± 7.4 years) participated in the tests; each wore a long-sleeved shirt buttoned at the wrist, long trousers, and running shoes without socks. A mesh jacket (BugOut Outdoor Wear, Wautosa, Wisconsin, USA) was worn over the head and arms and surgical gloves were worn on the hands; the legs of the trousers were rolled up to the knee to expose only the lower legs to biting mosquitoes.

One milliliter of test repellent was applied to each leg of 2 volunteers with the use of a pipette (Gilson, France). The repellent was spread evenly over the leg from the base of the knee to the ankle. The amount of repellent per square centimeter varied among volunteers because of leg-size differences. The approximate application area [$A = 1/3(a + b + c) \times h$] was calculated from measurements of leg length (h, knee to the ankle) and circumference (a, just below the knee; b, the calf; and c, the ankle). Based on these leg-size areas, the compound doses were 0.2–0.3 mg/cm^2 skin. Both legs of a 3rd volunteer were treated with 1 ml ethanol per leg, and this person served as an untreated control.

The repellent compounds were evaluated against mosquitoes on each of 3 nights and applied under supervision at 1730 h on each night 2 h before the start of each test at 1930 h. The volunteers entered the test area, sat in predetermined positions approximately 5 m apart, and collected all mosquitoes biting in 10 min, followed by a 50-min break. Mosquitoes were collected by the untreated control and by repellent-treated volunteers with aspirators and placed into containers covered with netting. This procedure was repeated hourly for 6 h, to obtain 6 biting collections by each volunteer. Tests were replicated and randomized so that all volunteers evaluated each treatment on 1 occasion, and were untreated control once.

The nightly mosquito collection totals at each of the hourly time intervals were determined for the controls and for each repellent treatment group. Percentage protection was calculated at each time interval by comparing the number of bites for controls against the number of bites for repellent-treated test participants with the use of Abbott's formula (Abbott 1925).

Percentage protection, defined as the number of bites received by an individual in a treatment group relative to that of the control, was calculated as (control minus treatment)/control × 100. Comparison of repellent efficacy was made among the 2 treatment groups with the use of a 2-way analysis of variance (ANOVA) with the repeated-measures method. The mean protection provided by the 2 repellent compounds was compared with the use of the Student-Newman-Kuels method ($P < 0.05$). Because the data were expressed as percentages (percentage protection), an arcsine transformation was performed on values before statistical analysis.

Table 1. Mean protection time for forearms treated with SS220 and deet against 3 species of mosquitoes in the laboratory.[1,2]

Chemical	Mean protection time[3] (±SE) for each mosquito species (min)		
	Anopheles farauti	*Aedes aegypti*	*Culex annulirostris*
20% SS220	18 ± 7.4 (n = 5)	150 ± 17.3 (n = 4)	180 (n = 3)
20% deet	82.5 ± 14.4 (n = 4)	195 ± 51.2 (n = 4)	>360 (n = 2)

[1] Mean probing rate on untreated forearm: *An. farauti* 7.2 ± 0.7/10 sec (n = 9), *Ae. aegypti* 7.6 ± 0.8/10 sec (n = 8), and *Cx. annulirostris* 4.8 ± 1.1/30 sec (n = 5).
[2] deet, *N*,*N*-diethyl-*3*-methylbenzamide; SS220, (1S, 2′S)-2-methylpiperindinyl-3-cyclohexen-1-carboxamide.
[3] Time until 3 bites were recorded.

RESULTS

Laboratory tests

The protection provided by each repellent against 3 species of mosquitoes is shown in Table 1. The tests showed that deet provided better protection in the laboratory than SS220 against all species.

Field test

The average area of the volunteers' legs protected was 1,334 cm^2 (range 950–1,656 cm^2) per leg and the amount of repellent applied to their legs was 0.2–0.3 mg/cm^2. A total of 494 mosquitoes from 8 species were collected, and the predominant species collected was *Cx. annulirostris* (Table 2). The overall mean biting rate of all mosquitoes on ethanol-treated (control) volunteers was 24.0 ± 5.2 bites per 10 min (Table 3). The mean number of mosquitoes collected throughout the collection period was fairly uniform, and there were no differences in the mean number of all mosquitoes (1-way ANOVA, $F_{5,12} = 0.81$, $P = 0.56$) and *Cx. annulirostris* (1-way ANOVA, $F_{5,12} = 0.78$, $P = 0.59$) collected each hour.

During the field efficacy trial, the percentage protection provided by the 2 repellent chemicals against all mosquitoes was significantly different (2-way ANOVA, $F_{1,10} = 8.8$, $P = 0.032$, Fig. 1). Deet provided >95% protection against all mosquitoes for only 2 h, compared with 5 h of protection by SS220. The percentage protection provided by the 2 repellents against *Cx. annulirostris* was not significantly different (2-way ANOVA, $F_{1,10} = 4.5$, $P = 0.09$, Fig. 2). However, the protection provided by 20% deet was only 55.8% 7 h after repellent application compared with 96.4% provided by 20% SS220 (Fig. 2).

Table 2. Number and species of mosquitoes collected at Redcliffe Airport, Queensland, Australia, January 2008.

Species	Number collected (%)
Anopheles annulipes	2 (0.4)
Aedes alternans	3 (0.6)
Aedes procax	3 (0.6)
Aedes vigilax	56 (11.3)
Aedes vittiger	1 (0.2)
Culex annulirostris	417 (84.4)
Culex australicus	3 (0.6)
Culex sitiens	9 (1.8)
Total	494 (100)

DISCUSSION

The results of this study have shown that SS220 provides good protection against mosquitoes in Queensland, Australia. The laboratory tests showed that deet provided better protection than SS220 against all species tested. In an earlier laboratory study conducted with racemic piperidine compound, AI3-37220 [1-(3-cyclohexen-1-yl carbonyl)-2-methylpiperidine], deet provided longer protection against *An. farauti* than AI3-37220 (Frances et al. 1998). In previous laboratory studies, the protection provided against *Cx. annulirostris* was longer than that provided against *Ae. aegypti* and least against *An. farauti* (Frances et al. 2005). In the current tests, protection provided by both deet and SS220 against different species followed the same pattern as previously observed.

In the field test, deet provided >95% protection for only 2 h, compared to 5 h provided by

Table 3. Mean (±SE) number of all mosquitoes and *Culex annulirostris* alone biting per 10 min on untreated (control) volunteers during hourly collections at Redcliffe Airport, Queensland, Australia, January 2008.

Time after repellent application (h)	Mean (±SE) number collected[1]	
	All mosquitoes (n = 432)	*Culex annulirostris* (n = 367)
2	8.3 ± 2.7 a	7.3 ± 2.7 a
3	14.7 ± 6.6 a	12.3 ± 6.4 a
4	25.7 ± 12.3 a	20.0 ± 11.1 a
5	23.3 ± 11.7 a	19.7 ± 10.4 a
6	41.7 ± 23.3 a	37.3 ± 21.8 a
7	30.3 ± 11.6 a	25.7 ± 9.8 a

[1] Means in the same row followed by the same letter are not significantly different with a 1-way ANOVA and Student-Newman-Kuels method ($P = 0.05$).

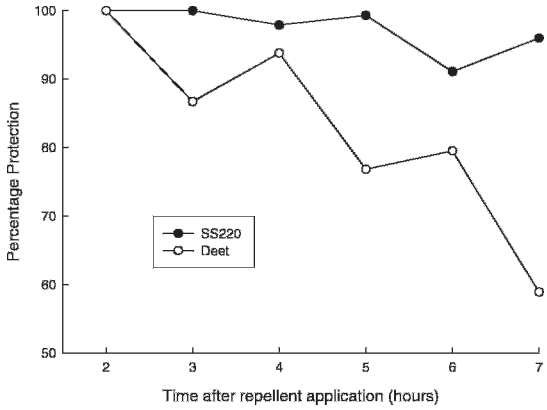

Fig. 1. Percentage protection provided by 20% deet (*N*,*N*-diethyl-*3*-methylbenzamide) and 20% SS220 [(1S, 2′S)-2-methylpiperindinyl-3-cyclohexen-1-carboxamide] against all mosquitoes at Redcliffe Airport, Queensland, Australia, in January 2008.

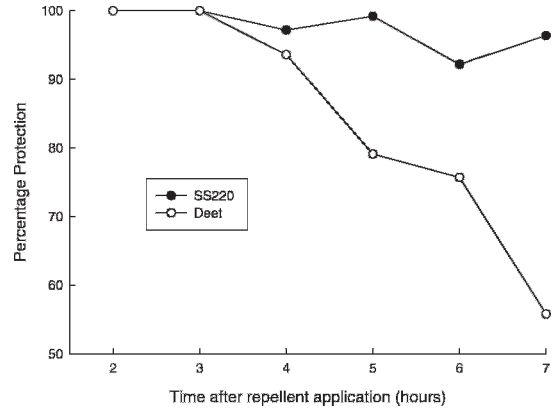

Fig. 2. Percentage protection provided by 20% deet (*N*,*N*-diethyl-*3*-methylbenzamide) and 20% SS220 [(1S, 2′S)-2-methylpiperindinyl-3-cyclohexen-1-carboxamide] against *Culex annulirostris* at Redcliffe Airport, Queensland, Australia, in January 2008.

SS220. We have no plausible explanation as to why SS220 was more effective than deet in field tests, but less effective in laboratory tests. The dose rates of repellent applied to the forearm in laboratory tests (0.4 mg/cm^2) was more than the application of the same repellents to volunteer legs (0.2–0.3 mg/cm^2) in the field tests. Repellent tests are used to compare active ingredients or formulations with the same standard tests, and variations in repellent assays occur because of both abiotic and biotic factors (Barnard et al. 2007).

Previous field studies have shown that AI3-37220 (racemic SS220) provided similar or better protection than deet against mosquitoes in Australia and Papua New Guinea (Frances et al. 1998, 1999, 2001). The results of the current study show that SS220 also provided substantially better protection than deet against mosquitoes in the field in southeast Queensland. Additional field studies to investigate the protection provided by SS220 against other natural populations of mosquitoes, primarily *Anopheles* sp., are warranted.

The formulation of repellent active ingredients can enhance the protection provided by repellents (Debboun et al. 2007). Future studies of formulations of SS220 should be conducted to determine if protection could be enhanced.

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